

How In Vitro Assays Contribute to Allergy Diagnosis

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Abstract Diagnosis of allergic disorders is based upon the clinical history of the disease, the immunoglobulin E (IgE) antibody response, and the allergen exposure. During the last decade, many changes have occurred in the in vitro diagnostic tests used in daily practice. The most important one is the use of allergenic molecules, which helps to define severe profile of allergy and/or to better understand cross-reactivity. The correlation between IgE sensitization and bronchial or nasal response in provocation tests is not so clear, which implies that such tests are still helpful in allergy diagnosis. In order to strengthen the link between a real allergen exposure and allergic symptoms, environmental allergen load assessment can be performed. For clinicians, it appears obvious to know the pollen count to treat their patients; however, they rarely measure the allergen load in the indoor environment, while nowadays home-tests (semi-quantitative or quantitative) make the assessment very easy. In the future, assessment of the environmental exposure (preferably with an indoor technician) of an allergic patient should take into account not only the allergens but also the other indoor pollutants, which could enhance respiratory symptoms in allergic patients.

Keywords Inhalant allergens · IgE · Diagnosis · Molecular allergology · Environmental assessment

Introduction

Diagnosis of allergic disorders (immediate or type I hypersensitivity) is based upon the clinical history of the disease, immunoglobulin E (IgE) antibody response, and evaluation of the allergen exposure. During the last two decades, progress in biochemistry and molecular biology enabled the characterization, cloning, recombinant production, and purification of allergen proteins or components allowing the detection and quantification of IgE antibodies to these proteins or components [1]. Those allergen molecules enabled the development of in vitro assays expanding the possibilities for the targeted allergen-specific diagnostics of IgE and the elaboration of purified allergen standards for environmental allergen exposure assessments as well [2, 3]. This report presents an overview of the clinical immunology laboratory assays and allergen exposure assessments and examines how those tests contribute to the diagnosis of patients with a disease associated with type I hypersensitivity to the most important inhalant allergens.

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Diagnostic Approach for Allergic Disease

A thorough clinical history investigation and physical examination constitute the first step of allergy diagnosis. The presence of symptoms suggesting a diagnosis of asthma, allergic rhinitis, or allergy skin disease should match with suspected relevant allergen exposures. Once the clinical history has been collected, many tests are available to detect allergen-specific IgE in the skin or

blood [4]. Skin testing is one of confirmatory tests for allergen-specific IgE antibody detection, which are used in clinical practice, with serum-based IgE antibody assays if necessary. Results from those tests in combination with the clinical history are usually sufficient to diagnose allergy to common inhalant allergens. In cases of discrepancy, the basophil activation test can be helpful for the diagnosis in specific cases, such as patients with local allergic rhinitis, and furthermore in vivo provocation tests can provide critical information [5–7].

Laboratory IgE Antibody Assays

In the beginning, specific IgE evaluation was based upon the detection and quantification of IgE antibodies directed against crude natural extracts. Those extracts consist of a mixture of allergenic and non-allergenic molecules such as proteins, polysaccharides, and lipids extracted from well-defined sources (e.g. house dust mites, animal dander,...). Many studies reported that different natural extracts vary in their allergen composition and potency [8–16]. Although manufacturers performed quality checks to guarantee better-characterized extracts and improved batch-to-batch consistency [17, 18], the extract-based testing cannot differentiate specific IgE sensitization, cross-reactivity between allergens, or identify the disease-eliciting allergenic molecules. Over the last decades, there have been developments of new methods to measure specific IgE antibodies alongside to a molecular characterization of allergen components. The traditional radio-immunological test (radioallergosorbent test, RAST) using radio-labeled anti-IgE antibodies has been replaced by a fluorescent immunoassay (FEIA) based on the “sandwich” principle and cellulose as solid-phase. Meanwhile, the availability of allergen molecules prepared by purification from native sources or molecular recombinant methods allows the determination of IgE-reactivity patterns at a molecular level and the concept of component resolved diagnostics has been proposed by R. Valenta [19].

The presence of IgE antibodies against allergen molecules can be determined using singleplex analysis (one assay per sample) or multiplex analysis (multiple assays per sample). Singleplex tests can be performed either with allergen extract or with allergen components. The indication for their use cannot be established in a standardized way but rather on a case-by-case basis and requires the clinician to select the allergen molecules necessary for an accurate diagnosis based on the clinical history of the patient, on the allergen source, and the availability of the single allergens. The molecular multiplex platforms are based on microarray technology and refer to the simultaneous determination of sIgE to different allergen components and therefore assess clinical sensitization patterns. Currently, the allergen microarray “ImmunoCAP® ISAC”

(Phadia AB, Uppsala, Sweden) based on the Immuno Solid-phase Allergen Chip is available for determining IgE and IgG responses toward 112 allergen molecules [20].

IgE Antibody Results and Clinical Relevance

Detection thresholds in specific IgE determination define the IgE antibody positivity as an indicator of sensitization. Historically, the lower detection threshold for specific IgE quantification was 0.35 kU_A/L. Clinical performance of ImmunoCAP Specific IgE tests has been evaluated in numerous studies and, as example, two studies performing specific IgE tests with whole-allergen extract reported a sensitivity of 87 and 90.9 % for cat dander and 88.5–90.9 % for *Dermatophagoides pteronyssinus*, respectively [21, 22]. In recent years, the analytical sensitivity of the IgE tests has improved and the lower limit of quantification (LoQ) is now 0.1 kU_A/L for singleplex autoanalyzers. The use of variable positive thresholds is frequent in research studies and may lead to debate on the most appropriate choice [23–25]. Nevertheless, in daily practice, a positive allergen-specific IgE antibody result should be reported as an analytical measurement down to the LoQ of 0.1 kU_A/L [26]. Improvement in the analytical sensitivity by lowering the assay’s LoQ should enhance the diagnostic sensitivity for sensitization determination. Therefore, evaluation of the clinical relevance of in vitro IgE tests has been a concern for more than 20 years and Receiver Operating Characteristic (ROC) analysis has been proposed to identify IgE decision levels defined as cut-off points [27]. The ROC curve offers a graphical illustration of the reciprocity of diagnostic sensitivity (true positives) and specificity (true negatives), and the difficulty to determine the optimum cut-off, which is a general feature of diagnostic tests [28]. ROC analysis, performed with cat- and dog-specific IgE measurements from the selected teenagers of the suburban Detroit childhood cohort reporting symptoms when exposed to pets, showed that clinical diagnostic of allergy was optimum with cut-offs at 0.12 and 0.2 kU_A/L, respectively. Comparison with the traditional cut point of 0.35 kU_A/L provided evidence for the utility of cut-off points as low as 0.1 kU_A/L [29]. Many studies have performed ROC analysis in carefully selected populations and produced variable cut-off points with different positive and negative predictive values, rather study-specific and therefore not easily extrapolated to an individual [25, 30, 31•]. Nevertheless, the use of ROC analysis is still interesting to compare two specific IgE assays [21, 32].

Results with the multiplex ISAC 112 are expressed within a range of 0.3 to 100 ISAC Standardized Units for IgE (ISU-E) using a standard calibration curve, and a LoQ of 0.3 ISU-E is assumed for all 112 allergens. However, in clinical practice,

results are reported semiquantitatively divided into four different categories (negative, low-level-positive, moderately high, very high) (www.phadia.com). When comparing molecular and extract-based allergy diagnostics, ISAC multiplex test correlates well with ImmunoCAP singleplex results with correlation value from 0.68 to 0.92 regarding on the allergen [33]. Positive percent agreement values above 90 % were obtained for timothy grass pollen, birch pollen, and hazelnut, while hazel pollen and cat were above 80 %. In contrast, the positive percent agreement for *Dermatophagoides pteronyssinus* was the lowest with 60 %, indicating that some mite allergenic molecules relevant for some of the patients tested may be missing in the multiplex assay [33]. Nevertheless, to truly compare both test methods, this should be done at the molecular level with each method. A comparison of the multiplex ISAC 112 and singleplex analysis of specific IgE to grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5b, rPhl p 6, rPhl p 7, rPhl p 11, rPhl p 12, and nPhl p 4) showed statistically significant correlations of the results with all the allergens and values ranging from 0.70 to 0.96 (excluding rPhl p 7 from the analysis because of the too few sensitizations in the patient series) [34]. Some discrepancies were observed for some allergens, notably nPhl p 4, rPhl p 11, and rPhl p 12, underlying that the technology of the two systems is widely different and has to be taken into consideration. The allergen binding to the test surface, which differs between the two technologies, could be one factor influencing the sensitivity. The technology used in ImmunoCAP test involves excess of bound allergenic molecule for the detection of the corresponding sIgE in the patient serum. By contrast, on the microarray chip, allergens are applied in less than picogram quantities on a single spot, which correspond to a quantity up to 10,000,000 times less protein, depending on the molecular weight [35]. Therefore, it is inevitable that those differences lead occasionally to discrepancies between the outcomes.

Independently of the origin of the allergens, extracts, or molecules, and of the method used, singleplex or multiplex, the presence of allergen-specific IgE strictly indicates allergic sensitization [4]. In fact, the diagnostic test's performance leading to determine the clinical relevance of an IgE antibody assay remains based on the clinical history of the patient. Thus, a patient with a positive allergy history and a positive allergen-specific IgE can only be determined by the clinician to have a clinically relevant IgE result. The same argument can be applied to concordant negative results that exclude allergy and underlying sensitization. In case of positive allergen-specific IgE results with no clinical history, IgE results should be referred as clinically irrelevant (rather than false positive) since they are an indicator of IgE sensitization [31]. Results analysis from multiplex are very challenging for physicians and the interpretation demanding, as the detection of IgE should be evaluated in combination with the clinical history. Several databases and advanced Web platforms have been

developed to coordinate information on allergen and protein families and therefore facilitate clinicians' access to information needed to handle and interpret "allergomics" data [36]. Furthermore, the WAO-ARIA-GA²LEN consensus document and the molecular allergology user's guide of the European Academy of Allergology and Clinical Immunology have been elaborated in order to provide information and training to clinicians [31, 37].

Diagnosis of IgE-mediated clinical allergy is demonstrated by the correspondence between IgE sensitization results and symptom occurrence after exposure to sensitizing allergen, which can be performed experimentally. Therefore, relationship has been investigated between first-line confirmatory tests, including sIgE and SPT, and in vivo provocation tests. Most studies reported good correlation between early asthmatic response in bronchial challenge and sIgE against common inhalant allergens (tested with allergen extracts), unlike SPT for which discrepancies have been observed [38, 39]. Recently, IgE to nDer p 1 and/or rDer p 2 has been reported to be predictive of immediate asthmatic response to broncoprovocation [32]. Concerning clinical outcome of nasal challenge tests performed with grass pollen, no dose response relationship has been observed with sIgE [40, 41]. Nevertheless, correlation results obtained with house dust mite are conflicting and could depend on the allergen [42].

Molecular Allergy Diagnostics—Rationales and Benefits

We have selected three situations in which allergy diagnosis was improved by the use of in vitro test performed with allergen components. Molecular allergy (MA) diagnostics allows increasing test sensitivity when the allergen was under-represented or lacking in the natural extract. Improvement in the diagnosis of latex allergy has been achieved by addition of rHev b 5 to the diagnostic repertoire of latex allergens [43, 44]. The absence of this allergen in the extract was responsible of false negative IgE test result in more than 25 % of patients with allergic reactions [45]. Since commercialization in 2002 of the new ImmunoCAP® containing latex allergen extract plus rHev b 5, the sensitivity of the latex-specific IgE determination was increased from 76 % (latex extract without rHev b 5) to 90 % and test efficacy from 86.7 to 93.8 %, respectively [46]. The use of an ImmunoCAP® (for research use), consisted of the latex allergens rHev b 1, rHev b 5, rHev b 6.01, and rHev b 8, improved the efficacy to 90.6 %. Today, commercially available component-based singleplex tests have been completed with the rHev b 3, rHev b 9, and rHev b 11 allergens [47, 48].

Using MA diagnostics, subgroups of patients with severe asthma can be defined. In case of particular single allergens with special characteristics, such as IgE sensitization

associated with severe asthma, introducing single allergens in clinical IgE tests enables improvement in test selectivity, that is to say analytical specificity. It was recently described that sensitization to Der p 2 and Der f 2 is associated with severity of asthma in house dust mite allergic patients [49•]. Using a customized multiplexed microarray (HIFI Allergy), patterns of sensitization were assessed in different cohorts of an international population study [50]. Interestingly, they observed that 19–20 % of patients had specific IgEs against group 2 allergens but not group 1, whereas 6–7 % of them have IgEs reacting with group 1 but not group 2. Thus, multiplex analysis would allow significant discrimination between patients with severe asthma, particularly concerning patient sensitized to mite allergens from group 2. Similarly, it has been shown in a cross-sectional study with pet-sensitized patients that sensitization to Can f 3, Fel d 2, or Equ c 3 was associated with more severe respiratory symptoms [51•]. Furthermore, in allergy to furry animals, several studies have observed that sensitization to more than one component from the same animal was associated with more severe asthma [51•, 52, 53•].

MA diagnostics allows detection of cross-reactivity, which is one of the common causes of a lack of analytical specificity of the allergen extracts. Multiplex analysis, performed in Italian adult multi-sensitized patients with respiratory symptoms, detected elevated sIgE to profilins in 15 % of the patients [54]. MA diagnostics enable to identify the presence of sIgE to profilins in patients with plant allergy, which may explain both perceived multi-sensitization and oral allergy symptoms to fruits and vegetables in patients without sIgE against PR-10. Profilins have been shown to elicit IgE response with major geographical variations, from 23 to 44 % of pollen-allergic patients in some regions of Spain [55]. The respiratory symptoms induced by the inhaled protein are most frequently limited [56]. However, oral provocation in food allergic patients with low dose of purified profilins has been shown to induce severe reaction in grass pollen allergic patients living in geographic areas with high grass pollen exposure [57••]. MA diagnostics proved to be a useful tool for accurate diagnosis in patients sensitized to panallergens and to improve the interpretation of their clinical relevance [58•].

Aeroallergen Exposure Assessment

Diagnosis of allergic diseases lies on a compatible clinical anamnesis, IgE sensitization, and allergenic exposure. However, clinical relevance of positive skin prick tests varies greatly with geographical distribution, type of allergen, and chosen cut-off, calling for complementary diagnostic tests [59]. In order to strengthen the link between a real allergen exposure and allergic symptoms, environmental allergen load assessment has been developed.

Because pollens are preponderant allergens, airborne pollen concentrations have been monitored for the first time by Charles Blackley at the end of the nineteenth century and all over the world since the 1950s, drawing up pollen calendars that are useful for allergy diagnosis. Airborne particles are trapped by various sampling devices using gravimetric, impaction, or suction techniques. Counting and identifying pollen grains is then performed under optical microscopy [60]. Nowadays, exposure to outdoor airborne allergens is monitored through a wide network such as EAN (European Aeroallergen Network) that comprises more than 400 pollen traps spread over 38 countries mainly of Europe (www.ean-net.org). The database itself is the largest, non-commercial pollen data collection in the world with long time series and is a very useful tool for many studies like the EU-funded project HIALINE (Health Impacts of Airborne Allergen Information Network) [61]. However, pollen counts might not be completely representative of allergen exposure also because there are some major and minor allergens [62]. That is why the HIALINE study compared traditional pollen counts with specific allergen detection for example Bet v 1, the major allergen from birch pollen, using a 2-site binding assay based on monoclonal antibodies in an ELISA format. The detection limit of the ELISA was 0.7 ng/mL of Bet v 1 which equals 2.1 pollen/m³ with a variability of 17.4 % for the low control (1.9 ng/mL) and of 12.8 % for the high control (7.4 ng/mL) whereas the variability of pollen counts at the same location between three Hirst-type pollen traps was 23 %. The average European allergen release from birch pollen was 3.2 pg Bet v 1/pollen and did not vary much between the European countries. However, in all countries, a >10-fold difference in daily allergen release per pollen was measured which could be explained by long-range transport of pollen with a deviating allergen release [63••]. Furthermore, the pattern of Bet v 1 isoforms could also be determined using the 2D-SDS PAGE technique coupling with spectrophotometric analysis and the allergenicity of air samples could be tested using basophil degranulation test offering a new perspective for allergy diagnosis in the future. Monitoring the allergens themselves together with pollen in ambient air might be an improvement in allergen exposure assessment and a great help for allergy diagnosis [63••].

While pollen counts are widely used by allergists, monitoring of indoor environment is still poorly employed and a lot of progresses need to be done. Environmental tests are even not mentioned in the international guidelines for allergic airways disease management despite the fact that a sensitization is insufficient to ascertain the reality of an allergen exposure [64] and the fact that patient history may not be a good guide to many airborne indoor allergies especially to mites and cockroaches. There is a lack of understanding about the nature and sites of allergen exposure with such a great variability in allergen types and loads depending on geographical area,

temperature, humidity, and home characteristics supporting the use of these tests [65]. Indeed, environmental tests to detect major indoor allergens such as mites, cats and dogs, cockroaches, molds, and rodents have provided more quantitative data on the effective allergen exposure at home. Testing allows identification of the reservoirs of allergens in carpets, furnishing, and clothing. For example, house-to-house variability was demonstrated for mite allergens with no difference between dwellings of allergic patients and controls highlighting the importance of measuring mite allergens [66]. Plenty of examples could be given with cat allergens that could be present in some homes without cats, in schools and child care centers, and in public buildings and public transport [67–69]. Cockroach allergens also are found in school dust [70] and in about 20 % of homes in the USA that have no visible evidence of cockroach infestation [71, 72]. The evaluation of the overall exposure of a subject is of a great complexity and should include indoor pollutants, such as formaldehyde. The impact on bronchial response of a 30-min exposure to formaldehyde has been documented experimentally in mite-sensitized asthmatic subjects and the effects of chronic domestic exposure such as dry night cough, a marker of allergy in preschool children, have been reported in the PARIS birth cohort study [73, 74]. Moreover, performance of a cluster analysis on 315 dwellings of mite allergic patients with rhinitis and/or asthma suggested that co-exposure to allergens and chemicals leads more often to asthma than rhinitis (F. de Blay, personal data). Recently, the joint task force of the American Academy of Allergy, Asthma & Immunology (AAAAI) and American College of Allergy, Asthma & Immunology (ACAAI) published several practice parameters on environmental exposure assessments as part of the allergy practice [75–77]. Based on a systematic literature review, those practice parameters provided categories of evidence linking allergen exposure, and allergic sensitization and symptoms, but no specific recommendations regarding allergen exposure thresholds for health effects have been established.

Thus, the first indoor environmental tests were developed more than 30 years ago and are of two different types: semi-quantitative and quantitative tests. The gold standard for allergen detection remains the quantitative ELISA tests using monoclonal antibodies directed toward purified allergens. The advantages of ELISA are high throughput, accurate quantitation (sensitivity of 0.1 µg allergen/g dust), and defined specificity (low non-specific binding with approximately 0.08 absorbance units), by using mAbs to allergens of known molecular structure and allergenic importance with a good reproducibility (coefficients of variation (CV) on the estimated geometric means from the analytical results ranged between 61 and 93 %) [78, 79]. The limitations of ELISA are that dust extraction, sample preparation, and the time-consuming assays are confined to well-equipped

laboratories [65]. Efforts to improve standardization were initiated in 2001 with the CREATE project of the European Union [3, 80]. Recombinant allergens from birch, grass, olive pollen, and house dust mite were investigated to develop certified reference materials for allergenic products and validate methods for their quantification. This approach could improve the continuity of environmental exposure assessments. So far, two recombinant major allergens, rBet v 1 and rPhl p 5, were further characterized and included in the European Pharmacopeia [81]. Recently, the CREATE principles were applied to eight purified allergens and provided a single standard of eight purified allergens [82]. Thanks to ELISA tests, it was demonstrated for example that rodent allergen load was equally high in some inner-city homes as in the professional environment [83]. Moreover, the use of ELISA tests allowed to show that the exposure to Mus m 1 or Rat n 1 was dramatically lower in France than in houses in the USA [84]. This may explain the difference in prevalence in allergies to rodents between France and USA.

To educate and demonstrate the importance of indoor aeroallergens in allergic diseases, fast and simplified semi-quantitative detection techniques have been developed. At start, it was centered on assessing exposure to mites that are the major indoor allergens, in dust samples.

The first one, the Acares test was based on the detection of guanine in mite feces that contain mostly group 1 mite allergens. It is a colorimetric assay in which results are given in four different classes from 0 (least allergen quantity) to 3 (highest allergen quantity). For 10 µg of mite allergens per gram of dust, Acares test has a specificity of 89 % when it is of class 2 and 98.5 % when it is of class 3 [85]. However, the major weakness of the Acares test is the interpretation for class 1 that corresponds to an average quantity of 5.7 µg/g with a range going from 0.03 to 25 µg/g [86].

Other domestic tests using immunologic antigen-antibody reactions were further developed. The oldest one is ACLOTTEST, a dipstick test that uses rabbit polyclonal antibodies conjugated to colloidal dye that could detect house dust mite allergens in 30 to 60 min [87]. Results are graded according to the intensity of the pink color that develops and correlates with ELISA. DUSTSCREEN is an allergen detection test based on monoclonal antibodies (mAb) bound to nitrocellulose, which detects multiple allergens in a single assay with one dust sample. The nitrocellulose strip has six capture mAb “spots” for different allergens, which are detected by a cocktail of enzyme-labeled mAbs. This test can be quantified with a strip reader and shows also a good correlation with ELISA but it takes several hours to perform, and it is best suited for pharmacy, clinic, or office use where allergen levels on dust samples can be obtained the same day.

A real advance in the development of a true rapid test for mite allergen is the lateral flow technology and gold-labeled mAb directed against Der p 2 and Der f 2 antigens [88]. It has the advantage to recognize in a single test two different mite species *D. pteronyssinus* and *D. farinae* being not influenced by geographical variability of the mite fauna. Detection of group 2 mite allergens was validated by the good correlation between groups 1 and 2 in nine different cities using the ELISA technique ($r = 0.48$, $p < 0.001$) [88]. It is quite sensitive (detection limit of 100 pg) and gives results in 10 min [88]. The rapid test is fit for home use including a plastic dust device (MITEST dust collector) that samples a defined area (0.25 m^2) and allows dust to be collected and extracted in 2 min for a better reproducibility [89].

Mitey Checker, another chromato-immunoassay directed against Der 2 allergens, was developed in Japan and significantly correlates with the Der 2 measured by ELISA (Spearman's rank correlation coefficient: $r = 0.83$, $p < 0.01$). Moreover it was reported to have a good sensitivity and specificity even against Der 1 allergens, respectively 91.8 and 71.1 % for dust samples containing more than $2 \text{ } \mu\text{g/g}$ of dust and 85.7 and 79.5 % for samples containing more than $10 \text{ } \mu\text{g/g}$ of dust [90].

Recently, a new technology was developed for analyzing dust extracts with high throughput. Multiplex ARray for Indoor Allergens (MARIA) uses fluorescent multiplex array technology that allows the simultaneous detection of eight major allergens (dust mites Der p 1, Der f 1 and group 2, cat Fel d 1, dog Can f 1, rat Rat n 1, mouse Mus m 1, and German cockroach Bla g 2) in a single reaction well with significantly increased sensitivity ($< 0.1 \text{ ng/mL}$) and reproducibility (intra-assay CV $< 5 \%$ and inter-assay CV $< 25 \%$) when compared to the ELISA method [91]. Moreover, performance criteria for fluorescent multiplex array technology are reproducible within laboratories (94 % of concordance correlation coefficient were ≥ 0.90) and between laboratories (80 % of intra-laboratory results fell within a 10 % CV) providing standardized and consistent allergen measurements [92].

However, dust analyses might not be the exact reflect of personal allergen exposure. Therefore, attempts to monitor airborne indoor allergen levels were tried using conventional ELISA at first and then using ELISA with amplification systems like the polymeric streptavidin that could detect picograms of allergens [93–95]. Yet airborne detection of mite and cockroach allergens has proved difficult even with amplification systems [96]. To overcome this problem, the HALOGEN system was developed [97]. It is an intranasal personal sampling device which can be worn by the patient for several hours during normal domestic activities that enables inhaled particulate allergen to be directly visualized and measured. Inhaled allergens are deposited onto small plastic slides coated with allergen-specific mAb. The assay is sensitive because the extracted allergen is bound to the membrane at a high local

concentration within the minute area around each particle and so is easily detected by immunostaining. HALOGEN is capable of analyzing allergens and particles together, combining the advantages of morphological approaches and immunoassay.

In occupational allergy diagnosis, the detection of an allergen exposure is fundamental. Traditionally, airborne dust is collected on filters using pumps, the filters are eluted, and allergen content in the eluate is analyzed using immunoassays. Lastly, airborne particle monitoring could be improved through the use of the intranasal personal samplers [98, 99].

Recently, novel methods to determine the pattern of personal exposure to mite allergen bioaerosols over 24-h periods were developed [100]. A miniature time-based air-sampler and a miniature time-lapse camera were co-located close to the breathing zone of the subjects. Airborne particles, drawn into the sampler at 2 L/min via a narrow slot, were impacted onto the peripheral surface of a disk mounted on the hour-hand of either a 12- or 24-h clock motor [101•]. Disks were cut into subsamples corresponding to different categories of indoor or outdoor location, extracted, and analyzed for mite allergen Der p 1 by an amplified ELISA. Indoor domestic Der p 1 exposures accounted for 59.5 % of total exposure, whereas total in-bed-asleep exposure, which varied 80-fold between individuals, accounted overall for 9.85 % of total exposure, suggesting beds are not often the main site of exposure [101•].

In conclusion, progress in biochemistry and molecular biology enabled detection and quantification of specific IgEs to allergen protein components. Molecular allergy diagnostics provide arguments to discriminate between primary sensitization and cross-reactivity with inhalant allergen, to assess the individual risk of severity of an allergic reaction and to facilitate the selection of patients for allergen-specific immunotherapy. The clinical relevance of all IgE findings with in vitro methods of molecular allergy diagnostics depends on the presence of corresponding symptoms and should be assessed on the basis of the patient's clinical history. Finally, in a bottom-up approach, multiplex analysis opens up new horizons with a detailed molecular profile of the IgE repertoire of the allergic patient, which may predict future allergic symptoms. Nevertheless, the interpretation of MA diagnostic methods is demanding and underlines the need of training for clinicians to handle and interpret the data. As the data from the aerobiology monitoring stations are used in daily practice to match symptoms and suspected relevant pollen allergen exposure, assessment of the environmental indoor aeroallergens exposure should be part of the allergy practice as well. Moreover, in the future, assessment of the exposure of an allergic patient should take into account not only the allergens but also the other indoor pollutants, which could enhance respiratory symptoms in allergic patients.

Compliance with Ethical Standards

Conflict of Interest A. Casset, N. Khayath and F. de Blay declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent Concerning the authors' research cited in this paper, all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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- Of importance
- Of major importance

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